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In-vitro activity of antimicrobial agents against *Neisseria gonorrhoeae* in Brussels

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SUMMARY The minimum inhibitory concentrations (MICs) of 18 antimicrobial agents against 104 strains of *Neisseria gonorrhoeae* isolated in the Brussels area between January and October 1976 have been measured. The MICs for penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin showed a bimodal distribution. The second modus strains of cephalexin (MIC=6·25 μg/ml) were relatively resistant to penicillin G (MIC≥0·08 μg/ml). About 51% of all strains were relatively resistant to penicillin G, 40·5% to ampicillin (MIC≥0·16 μg/ml), 46% to amoxycillin, and 47·5% to carbenicillin. For cephalexin and cephaloridine, 25% and 8·5% respectively of all strains were relatively resistant (MIC >3·12 μg/ml). For cefazolin all MICs fell into a range of 0·097–3·12 μg/ml. Resistance to tetracycline, doxycycline, minocycline, erythromycin, and spiramycin (MIC ≥ 1μg/ml) was found in 9·5%, 7%, 6%, 36·5%, and 71% respectively of all isolates. No strains were resistant to rifampicin. For chloramphenicol and thiamphenicol the MICs ranged from 0·39 to 12·5 μg/ml and from 0·195 to 3·12 μg/ml respectively. The results for sulphamethoxazole, trimethoprim, and the combination of sulphamethoxazole and trimethoprim in a 20:1 ratio are given and discussed. The fractional inhibitory concentration (FIC) indices have also been calculated. No β-lactamase-producing strains were found, and a contingency coefficient C has been determined for all the pairs of antibiotics investigated.

Introduction

Over the past 15 years a decrease of the *in-vitro* sensitivity of *Neisseria gonorrhoeae* to many antibiotics has been noticed. This evolution is well documented, especially in the English-language literature. Many authors have found a strong, positive rank correlation between the sensitivities of gonococcal strains to various antibiotics (Verhagen *et al.*, 1971; Niel *et al.*, 1971; Robson and Salit, 1972; Maness and Sparling, 1973; Givan and Keyl, 1974; Stolz *et al.*, 1974, 1975; Meheus *et al.*, 1976).

Some authors have suggested that there might be a common basis for resistance to various antibiotics (Sparling, 1972; Maness and Sparling, 1973). Recently, linked genetic loci of resistance have been demonstrated (Maier *et al.*, 1975; Sarubbi *et al.*, 1975; Sparling *et al.*, 1975).

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There are also regional differences in the sensitivity patterns of gonococci (Jaffe *et al.*, 1976), and the incidence of less sensitive strains can be affected by the introduction of strains from overseas by travellers (Wols-Van der Wielen, 1970; Silver and Darling, 1971; Stolz *et al.*, 1974, 1975) or by inappropriate antibiotic regimens.

Furthermore, many authors have found that treatment failures with different antibiotic regimens very often correlated with the presence of relatively resistant strains (Nicol *et al.*, 1968; Leigh *et al.*, 1969; Martin *et al.*, 1970; Jaffe *et al.*, 1976).

It is therefore important to continue carrying out surveys to determine the prevailing MICs, particularly since these are necessary for determining treatment schedules and for drawing up rational surveillance programmes.

Material and methods

GONOCOCCAL STRAINS

One hundred and four strains of *N. gonorrhoeae* were isolated from male and female patients attending

the clinic for venereal diseases at the St Pieters Hospital in Brussels between January and October 1976. The diagnosis was made by examination of the Gram-stained smears. The strains were isolated on selective Thayer-Martin medium. All cultures were incubated at 37°C in 10% CO_2 for 48 hours. The isolates were identified by colonial morphology, Gram-stained preparations, oxidase activity, and sugar fermentation. The purified cultures were suspended in sterile horse serum and stored in liquid nitrogen until further investigation.

SENSITIVITY TESTING

Culture media

Antibiotic sensitivity testing was performed in the bacteriology laboratory at the Brugmann Hospital, using an agar dilution method. An inoculum containing 10^5–10^6 colony-forming units was inoculated on to a medium containing BHI-broth (Difco 0037-01) and 1·5% agar to which was added 10% defibrinated horse blood (Institut Pasteur, Brussels) and 10% sterile GC supplement (Oxoid, SR 56). The plates were incubated in 10% CO_2 for 48 hours. The level of the CO_2 was controlled by a Portomatic Auto CO_2 controller (Forma Scientific Inc. 3056/3062). The resulting colonies were suspended in a fluid medium of BHI-broth (Difco 0037-01) enriched with 5% of a haemin solution of 600 μg/ml (BDH-Biochemicals 24011) and 1% polyvitex (Bio-Merieux 5-5651) and incubated for 24 hours in 10% CO_2. These overnight cultures were then inoculated (using Steers' multipoint apparatus) on to plates containing twofold dilutions of the antibiotics. The medium used for testing the sensitivity of the organisms against the antimicrobial agents was GC agar (Difco 0289-01) plus haemoglobin (Difco 0136-01) plus 1% polyvitex (Bio-Merieux 5-5651). For testing the sensitivity of organisms against sulphamethoxazole, trimethoprim, and co-trimoxazole the medium used was GC agar (Difco 0289-01) plus 5% haemolysed horse blood (Institut Pasteur, Brussels) plus 1% polyvitex (Bio-Merieux 5-5651).

In each series one control plate without any antibiotic was included. In each run a *Staphylococcus aureus* (ATCC25923) and an *Escherichia coli* (ATCC 25922) with known sensitivity patterns for the antimicrobial agents were tested simultaneously. The plates were incubated in 10% CO_2 and read after 48 hours. The lowest antibiotic concentration to inhibit growth completely, or almost completely, was regarded as the minimum inhibitory concentration (MIC). A faint haze of growth or a single colony was disregarded.

Antimicrobial agents

The following antimicrobial agents were tested: sodium penicillin G (RIT Genval), ampicillin, amoxycillin, and carbenicillin (Beecham Laboratories); cephaloridine, cephalexin, and cefazolin (Eli Lilly Laboratories); tetracycline HCl (CERTA); doxycycline (Pfizer Inc.); minocycline (Lederle); chloramphenicol and rifampicin (Lepetit); thiamphenicol (Zambon); erythromycin (Abbott Laboratories); spiramycin (SPECIA); sulphamethoxazole and trimethoprim (Roche SA); and a combination of sulphamethoxazole and trimethoprim in a 20:1 ratio.

Screening for penicillinase-production was performed with a chromogenic cephalosporin (Glaxo compound 87/312).

Results

The distribution of the MICs of the 18 antimicrobial agents for all the isolates is shown in Table 1. The MIC distributions for penicillin G, ampicillin, amoxycillin, and carbenicillin show a bimodal distribution. For each drug there was a difference between the number of strains inhibited at the two peaks, such that when the two were compared by the χ^2 test $P<0{\cdot}001$ (Figure).

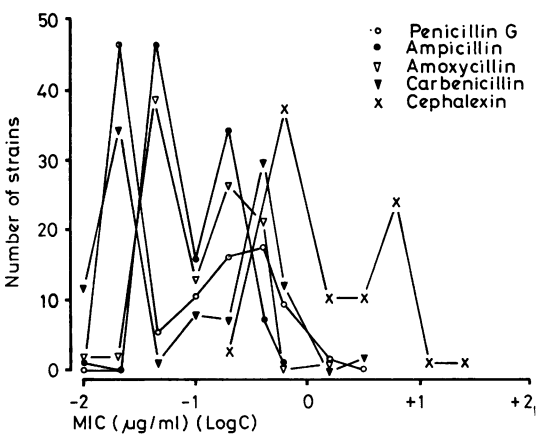

Figure *Distribution of MICs*

For penicillin G we found a first peak at 0·195-0·39 μg/ml. Relative resistance (RR) may be defined as a MIC ⩾0·08 μg/ml. The proportion of relatively resistant strains for penicillin G was 51%, and 26% of the strains were classified as having a 'high level' resistance to penicillin G (MIC ⩾0·39 μg/ml). One strain required 1·56 μg/ml of penicillin G for inhibition. Both ampicillin and amoxycillin showed a peak at 0·048 μg/ml and a second one at 0·195 μg/ml. Of all the strains 40·5% were relatively resistant to ampicillin and 46% to amoxycillin (MIC ⩾0·16 μg/ml). Carbenicillin had a first peak at 0·024 μg/ml and a second at 0·39 μg/ml; 47·5% of the isolates were relatively resistant.

Table 1 *Distribution of MICs of* 18 *antimicrobial agents against* 104 *strains of gonococci*

	MIC (μg/ml)														
Antimicrobial agent	*0·012*	*0·024*	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	*>100*
Penicillin G		46	5	10	16	17	9	1							
Ampicillin	1		46	15	34	7	1								
Amoxycillin	2	2	39	13	26	21		1							
Carbenicillin	12	34	1	8	7	29	12		1						
Cephaloridine							11	52	32	9					
Cephalexin					2	19	37	10	10	24	1	1			
Cefazolin				13	39	15	22	12	3						
Tetracycline					28	44	22	8	2						
Doxycycline				3	40	34	20	2	5						
Minocycline				7	38	43	10	5	1						
Chloramphenicol						53	26	8	10	5	2				
Thiamphenicol					8	68	11	13	4						
Erythromycin				1	10	36	19	27	6	5					
Spiramycin			1			2	27	22	31	11	8	2			
Rifampicin		2	5	30	46	16	5								

	0·24	*0·48*	*0·97*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*	*62·5*	*125*	*250*	*500*	*1000*	*2000*	*>2000*
Sulphamethoxazole		1	1	2	4	1	6	13	28	13	14	8	12		1

	0·024	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	*200*	*>200*
Trimethoprim		2									1	42	51	6	2

	Sulphamethoxazole									
Trimethoprim	*0·48*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*	*62·5*	*250*	*>2000*	
0·024	2									
0·097		3								
0·195			5							
0·39				9						
0·78					33					
1·56						28				
3·12							22			
12·5								1		
>100									1	

Ampicillin was slightly more active than amoxycillin and penicillin G; carbenicillin was less active.

Among the cephalosporins only cephalexin showed a bimodal distribution with a first modus at 0·78 μg/ml and a second at 6·25 μg/ml ($P<0·001$) (Figure). The second modus strains of cephalexin were all relatively resistant to penicillin G, while 91·5% were relatively resistant to ampicillin and 96% to amoxycillin and carbenicillin (Table 2). The MIC for cephaloridine was greater than 3·12 μg/ml (RR level) in 8·5% of the strains compared with that in 25% for cephalexin. For cefazolin all MIC values fell into a range of 0·097–3·12 μg/ml, with a peak at 0·195 μg/ml. Cefazolin was the most active, while cephaloridine was slightly more active than cephalexin.

Among the tetracyclines, minocycline was the most active; tetracycline and doxycycline showed almost the same degree of activity. All the tetracyclines had a peak at 0·195–0·39 μg/ml. For doxycycline, tetracycline, and minocycline 7%, 9·5%, and 6% respectively of the isolates were relatively resistant (MIC $\geqslant$1 μg/ml).

Chloramphenicol and thiamphenicol both showed a peak at 0·39 μg/ml sloping off to the right. Thiamphenicol has a better *in-vitro* activity than chloramphenicol. For chloramphenicol 24% of the strains had a MIC $\geqslant$1 μg/ml compared with 16·5% for thiamphenicol.

Erythromycin was more active than spiramycin. The peak for spiramycin was block-shaped, and the MICs ranged from 0·78–3·12 μg/ml. For erythromycin 36·5% of the strains were resistant (MIC $\geqslant$1 μg/ml); for spiramycin 71% were resistant. For rifampicin all MICs fell into a range of 0·024–0·78 μg/ml.

The range of MICs was very wide for sulphamethoxazole, ranging from 0·48–1000 μg/ml. One strain required more than 2000 μg/ml for inhibition. The MIC distribution for trimethoprim was characterised by a peak at the right side of the curve, with values ranging from 50–100 μg/ml. Two strains were very sensitive (MIC=0·048 μg/ml). The 20:1 combination of sulphamethoxazole and trimethoprim resulted in a reduction of the MIC values. We have also calculated the fractional

Table 2 *MICs for the penicillins for the second modus strains of cephalexin (MIC=6·25 μg/ml)*

	MIC (μg/ml)			
No. of strains	*Penicillin G*	*Ampicillin*	*Amoxycillin*	*Carbenicillin*
2	0·78	0·195	0·195	0·195
6	0·195	0·195	0·39	0·78
19	0·78	0·39	0·39	0·78
29	0·195	0·097	0·097	0·39
34	1·56	0·39	0·39	0·024
45	0·195	0·097	0·195	0·39
46	0·39	0·195	0·39	0·78
51	0·195	0·195	0·39	0·39
53	0·39	0·195	0·39	0·78
54	0·195	0·195	0·195	0·39
79	0·39	0·39	0·39	0·78
86	0·39	0·195	0·195	0·39
87	0·39	0·195	0·39	0·39
92	0·195	0·195	0·195	0·39
98	0·39	0·195	0·39	0·39
110	0·39	0·39	0·39	0·78
111	0·097	0·195	0·195	0·39
113	0·78	0·78	1·56	3·12
136	0·195	0·195	0·195	0·39
140	0·195	0·195	0·195	0·39
153	0·39	0·195	0·195	0·39
G1	0·78	0·195	0·39	0·78
G9	0·39	0·195	0·195	0·39
G1B	0·78	0·195	0·195	0·39

inhibitory concentration (FIC) indices (Table 3). The FIC index is the sum of the fractional inhibitory concentration of both drugs. This is an expression of the synergy between sulphamethoxazole and trimethoprim. The lower the index the greater is the synergy.

Screening for penicillinase-producing strains was carried out with a chromogenic cephalosporin. We did not find any β-lactamase-positive strains.

The number of strains that showed cross-resistance with penicillin G is shown in Table 4. Cross-resistance was pronounced for the other penicillins, cephalexin, erythromycin, spiramycin, chloramphenicol, and sulphamethoxazole. We found four multiresistant strains, that is, strains which were resistant for all the antimicrobial agents, except cefazolin and rifampicin.

The contingency coefficient C, computed from a 2×2 contingency table for all pairs of antimicrobial agents, is shown in Table 5. The significance of the factor C is tested by the χ_2 test. The contingency coefficient C is a measure of the extent of association or relation between two sets of attributes. A degree of correlation was found in the group of penicillins and, individually, between cephalexin, chloramphenicol, thiamphenicol, and the penicillins.

Table 3 *FIC index for all isolates**

	Isolates	
FIC index	*No.*	*%*
<0·25	35	33·7
>0·25–0·50	28	26·9
>0·50–0·75	31	29·8
>0·75–1·00	0	0
>1·00	10	9·6

$$*\text{FIC index} = \frac{\text{TMP in combination}}{\text{TMP alone}} + \frac{\text{SMZ in combination}}{\text{SMZ alone}}$$

Discussion

Since the late 1950s the proportion of gonococci that are less sensitive to penicillin G has increased,

Table 4 *Number of strains relatively resistant (RR) to penicillin G which show cross-resistance with other antibacterial agents**

	Strains	
Antibacterial agent	*No.*	*%*
Ampicillin	41	77·4
Amoxycillin	47	88·7
Carbenicillin	47	88·7
Cephaloridine	9	17·0
Cephalexin	26	49·1
Cefazolin	0	0
Tetracycline	9	17·0
Doxycycline	7	13·2
Minocycline	6	11·3
Chloramphenicol	22	41·5
Thiamphenicol	17	32·1
Erythromycin	27	51·0
Spiramycin	40	75·5
Rifampicin	0	0
Sulphamethoxazole	41	77·4
Co-trimoxazole	15	28·3

*53 strains relatively resistant to penicillin G.

Table 5 *Contingency coefficient C, computed from a 2×2 contingency table, for all pairs of antimicrobial agents (significance by χ^2 test)*

	PEN	*AMP*	*AMX*	*CAR*	*CRD*	*CLX*	*CZL*	*TET*	*DOX*	*MIN*	*CHL*	*THI*	*ERY*	*SPI*	*RIF*	*SMZ*	*SXT*
PEN																	
AMP	0·61‡																
AMX	0·66‡	0·66‡															
CAR	0·65‡	0·64‡	0·68‡														
CRD	0·28†	0·34‡	0·32‡	0·17§													
CLX	0·49‡	0·52‡	0·50‡	0·47‡	0·41‡												
CZL	0.00§	0·00§	0·00§	0·00§	0·00§	0·00§											
TET	0·24†	0·32‡	0·28†	0·20*	0·44‡	0·39‡	0·00§										
DOX	0·24†	0·32‡	0·28†	0·20*	0·59‡	0·35‡	0·00§	0·57‡									
MIN	0·24*	0·28†	0·26†	0·17§	0·55‡	0·32‡	0·00§	0·53‡	0·68‡								
CHL	0·39‡	0·45‡	0·42‡	0·39‡	0·30†	0·33‡	0·00§	0·45‡	0·44‡	0·40‡							
THI	0·40‡	0·44‡	0·44‡	0·39‡	0·39‡	0·42‡	0·00§	0·55‡	0·52‡	0·49‡	0·17§						
ERY	0·28†	0·36‡	0·32‡	0·28†	0·37‡	0·14§	0·00§	0·22*	0·33‡	0·32‡	0·42‡	0·30†					
SPI	0·10§	0·17§	0·08§	0·09§	0·20*	0·03§	0·00§	0·13§	0·17§	0·14§	0·20*	0·17§	0·40‡				
RIF	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§		
SMZ	0·10§	0·10§	0·04§	0·05§	0·17§	0·14§	0·00§	0·05§	0·08§	0·05§	0·14§	0·03§	0·05§	0·09§	0·00§		
SXT	0·13§	0·11§	0·09§	0·08§	0·37‡	0·24†	0·00§	0·05§	0·22*	0·24†	0·17§	0·13§	0.24*	0.10§	0·00§	0·26†	

* $6{\cdot}64 > \chi^2 > 3{\cdot}84$ $\quad 0{\cdot}01 < P < 0{\cdot}05$
† $10{\cdot}83 > \chi^2 > 6{\cdot}64$ $\quad 0{\cdot}001 < P < 0{\cdot}01$
‡ $\chi^2 > 10{\cdot}83$ $\quad P < 0{\cdot}001$
§ $\chi^2 < 3{\cdot}84$ $\quad P > 0{\cdot}05$

PEN=penicillin G; AMP=ampicillin; AMX=amoxycillin; CAR=carbenicillin; CRD=cephaloridine; CLX=cephalexin; CZL=cefazolin; TET=tetracycline; DOX=doxycycline; MIN=minocycline; CHL=chloramphenicol; THI=thiamphenicol; ERY=erythromycin; SPI=spiramycin; RIF=rifampicin; SMZ=sulphamethoxazole; SXT=co-trimoxazole.

and the distribution of MICs has become bimodal with one peak for the sensitive population and another one for the less sensitive strains. A review of the proportion of strains with decreased sensitivity in different countries is shown in Table 6. The lowest proportion of relatively resistant strains occurs in Western Europe, while the highest proportion is in the developing countries.

We found that 51% of the strains were relatively resistant to penicillin G, and this is a higher percentage than that reported by Meheus *et al.* (1976), in a former Belgian study. We found that 40·5% of our strains were relatively resistant to ampicillin, and this agrees well with the results reported by Meheus *et al.* (1976) and by Stolz *et al.* (1974) in the Netherlands.

We found that the proportion of strains relatively resistant to amoxycillin was 46%. In an evaluation of amoxycillin in the treatment of gonorrhoea in the USA, Karney *et al.* (1974) reported a figure of 39%.

Reports of the *in-vitro* sensitivity of gonococci to carbenicillin are very few. Karney *et al.* (1974) reported 43% of strains as relatively resistant (MIC $\geqslant$0·16 μg/ml); we found a figure of 47·5%.

In our study the bimodal distribution for cephalexin was noteworthy. All the second modus strains were relatively resistant to penicillin G. Some authors have found a high *in-vitro* resistance to cephaloridine. Robson and Salit (1972) in Canada reported 63% of the strains with a MIC $\geqslant$4 μg/ml. Givan and Keyl (1974) in Canada reported that 36%, 28%, and 10% of the cultures were resistant to 5 μg/ml of cephaloridine in 1971, 1972, and 1973 respectively. We found only 8·5% of the strains with a MIC >3·12 μg/ml.

Cefazolin is the most active of the cephalosporins. We found that 9·5% of our strains were relatively resistant to tetracycline. This is a very low proportion compared with that found by others (Reyn, 1969; Verhagen *et al.*, 1971; Niel *et al.*, 1971; Robson and Salit, 1972; Givan and Keyl, 1974; Stolz *et al.*, 1974, 1975; Jaffe *et al.*, 1976; Meheus *et al.*, 1976). We noticed that the degree of *in-vitro* resistance to tetracycline did not equal that to penicillin G.

For erythromycin and spiramycin 36.5% and 71% respectively of the strains had a MIC $\geqslant$1 μg/ml. Meheus *et al.* (1976) found their population was very sensitive to erythromycin; no strain had a MIC greater than 1 μg/ml. Robson and Salit (1972) reported 24% of the strains with a MIC $\geqslant$1 μg/ml, and Givan and Keyl (1974) found 19%. Niel *et al.* (1971) in France described an increasing proportion of resistant strains to spiramycin, especially after 1967.

We have found no strains resistant to rifampicin, and our results compare with those of Meheus *et al.* (1976) and Stolz *et al.* (1975).

For sulphamethoxazole 73% of the strains had a MIC greater than 50 μg/ml. Meheus *et al.* (1976) found only 37% of the strains were resistant.

Synergy between sulphamethoxazole and trimethoprim can be expressed by the FIC index. Of our

Table 6 *Evolution of the prevalence of strains of* Neisseria gonorrhoeae *relatively resistant to penicillin G*

Authors	*Date of publication*	*Country*	*Year of study*	*No. of strains*	*Decreased sensitivity*	
					Prevalence (%)	*MIC*
Nicol *et al.*	1968	England	1966	98	37·3	⩾0·1 μg/ml
Leigh *et al.*	1969	England	1968	189	40	⩾0·1 iu/ml
Reyn	1969	SE Asia, Western-Pacific	1961	44	64	⩾0·1 μg/ml
Reyn	1969	SE Asia, Western Pacific	1967–68	43	90	⩾0·1 μg/ml
Gray *et al.*	1970	England	1968–69	517	35	⩾0·06 μg/ml
Martin *et al.*	1970	USA	1945–54	771	1	⩾0·1 iu/ml
Martin *et al.*	1970	USA	1965	1124	42	⩾0·1 iu/ml
Martin *et al.*	1970	USA	1968–69	649	65	⩾0·1 iu/ml
Wols-Van der Wielen	1970	Netherlands	1968–69	216	35	⩾0·1 iu/ml
Niel *et al.*	1971	France	1958–66	569	10	>0·1 iu/ml
Niel *et al.*	1971	France	1967–68	66	19·7	>0·1 iu/ml
Niel *et al.*	1971	France	1969	100	31	>0·1 iu/ml
Silver and Darling	1971	England	1968–69	95	50·5	>0·1 μg/ml
Verhagen *et al.*	1971	Kenya	1968–69	736	65·1	⩾0·1 iu/ml
Robson and Salit	1972	Canada	1970–71	100	61	⩾0·1 iu/ml
Maness and Sparling	1973	USA	1971	147	69	⩾0·06 μg/ml
Stolz *et al.*	1974	Netherlands	1971–72	430	37	⩾0·08 μg/ml
Shahidullah and Greaves	1975	England	1975	300	30	⩾0·1 μg/ml
Meheus *et al.*	1976	Belgium	1974	105	42	⩾0·08 μg/ml
Vanhoof *et al.* (present study)	1978	Belgium	1976	104	51	⩾0·08 μg/ml

104 isolates 63 had a FIC index <0·5. Of those 53 strains resistant to sulphamethoxazole all were sensitive to co-trimoxazole; of these 41 had a FIC index <0·5 and 10 an index of less than 0·10 (Table 7). For co-trimoxazole 24 strains were resistant to 50 μg/ml of the mixture, even though 18 of these strains had a FIC index <0·5. In all, 10 strains had a FIC index >1. Four strains had a FIC index >2, which indicates a certain degree of antagonism.

We did not find any β-lactamase-positive strains. The first β-lactamase-producing gonococcus isolate in Belgium was recently reported by Piot (1977) from Antwerp. The patient was a Causasian heterosexual man who had been in the Ivory Coast.

We did not find any significant cross-resistance between penicillin G and tetracycline, although this has been noticed by many authors (Niel *et al.*, 1971; Verhagen *et al.*, 1971; Robson and Salit, 1972).

Table 7 *FIC index of co-trimoxazole sensitivity*

Sensitivity	*No. of strains*		*FIC index*	
			<0·5	*⩾1*
Co-trimoxazole-sensitive	80			
Sensitive to sulphamethoxazole		27	4	7
Resistant to sulphamethoxazole		53	41	0
Co-trimoxazole-resistant	24			
Sensitive to sulphamethoxazole		1	18	2
Resistant to sulphamethoxazole		23	0	1
Total	104	104	63	10

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